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L8 ANSWER 1 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

ACCESSION NUMBER: 2000:75786 BIOSIS
DOCUMENT NUMBER: PREV200000075786
TITLE: Transferrin-liposome-mediated systemic p53 gene therapy in combination with **radiation** results in regression of human head and neck cancer xenografts.
AUTHOR(S): Xu, Liang; Pirollo, Kathleen F.; Tang, Wen-Hua; Rait, Antonina; Chang, Esther H. (1)
CORPORATE SOURCE: (1) Lombardi Cancer Center, Georgetown University Medical Center, 3970 Reservoir Road NW, Research Building/E420, Washington, DC USA
SOURCE: Human Gene Therapy, (Dec. 10, 1999) Vol. 10, No. 18, pp. 2941-2952.
ISSN: 1043-0342.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The use of **cationic liposomes** as nonviral vehicles for the delivery of therapeutic molecules is becoming increasingly prevalent in the field of gene therapy. We have previously demonstrated that the use of the transferrin ligand (Tf) to target a **cationic liposome** delivery system resulted in a significant increase in the transfection efficiency of the complex (Xu, L., Pirollo, K.F., and Chang, E.H. (1997). Hum. Gene Ther. 8, 467-475). Delivery of wild-type (wt) p53 to a **radiation**-resistant squamous cell carcinoma of the head and neck (SCCHN) cell line via this ligand-targeted, liposome complex was also able to revert the **radiation** resistant phenotype of these cells in vitro. Here we optimized the Tf/liposome/DNA ratio of the complex (LipT) for maximum tumor cell targeting, even in the presence of serum. The efficient reestablishment of wtp53 function in these SCCHN tumor cells in vitro, via the LipT complex, restored the apoptotic pathway, resulting in a significant increase in **radiation**-induced apoptosis that was directly proportional to the level of exogenous wtp53 in the tumor cells. More significantly, intravenous administration of LipT-p53 markedly sensitized established SCCHN nude mouse xenograft tumors to radiotherapy. The combination of systemic LipT-p53 gene therapy and **radiation** resulted in complete tumor regression and inhibition of their recurrence even 6 months after the end of all treatment. These results indicate that this tumor-specific, ligand-liposome delivery system for p53 gene therapy, when used in concert with conventional radiotherapy, can provide a new and more effective means of cancer treatment.

L8 ANSWER 2 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5

ACCESSION NUMBER: 1998:47269 BIOSIS
DOCUMENT NUMBER: PREV199800047269
TITLE: Antisense raf oligodeoxyribonucleotide is protected by liposomal encapsulation and inhibits Raf-1 protein expression in vitro and in vivo: Implication for gene therapy of radioresistant cancer.
AUTHOR(S): Gokhale, P. C.; Soldatenkov, V.; Wang, F.-H.; Rahman, A.; Dritschilo, A.; Kasid, U. (1)
CORPORATE SOURCE: (1) E208 Res. Build., Lombardi Cancer Cent., 3970 Reservoir Rd. NW, Washington, DC 20007 USA
SOURCE: Gene Therapy, (Dec., 1997) Vol. 4, No. 12, pp. 1289-1299.
ISSN: 0969-7128.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We have redesigned **cationic liposomes** by using a combination of dimethyldioctadecyl ammonium bromide, phosphatidylcholine and cholesterol to enhance the in vitro and in vivo effectiveness of antisense raf oligodeoxyribonucleotide (ODN). Circulating ODNs carried in vivo by liposomes were intact for at least 24 h, while free ODNs were

undetectable after 5 min. Liposome-encapsulated antisense raf ODN (LE-ATG-AS) inhibited Raf-1 protein expression in vitro and in vivo. Furthermore, radioresistant tumor cells treated with LE-ATG-AS raf ODN were sensitized to ionizing **radiation**. These data provide new information for the delivery and potency of antisense ODN in vivo, and support the use of LE-ATG-AS raf ODN for gene therapy of radio-resistant cancer.

L8 ANSWER 3 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7

ACCESSION NUMBER: 1997:167991 BIOSIS
DOCUMENT NUMBER: PREV199799474594
TITLE: In vitro studies of liposome-mediated gene transfer into head and neck cancer cell lines.
AUTHOR(S): Wollenberg, B. (1); Lang, S.; Schmitt, B.; Kastenbauer, E.; Zeidler, R.
CORPORATE SOURCE: (1) Dep. Oto-Rhino-Larngology, Univ. Munich, Grosshadern Med. Cent., Marchioninistrasse 15, D-81377 Munich Germany
SOURCE: European Archives of Oto-Rhino-Laryngology, (1997) Vol. 254, No. SUPPL. 1, pp. S130-S132.
ISSN: 0937-4477.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The 5-year survival rate of patients with squamous cell carcinoma of the head and neck (HNSCC) has remained poor despite innovative surgery and new **radiation** and chemotherapeutic strategies. In such patients, gene therapy relying on the modification of tumor cells by gene transfer may have great potential as a new treatment modality in the therapy of HNSCC. In the present study we developed an in vitro model to show the efficacy and technical feasibility of **cationic liposome** -mediated gene transfer into HNSCC. Five adherent squamous cell carcinoma cell lines were transfected with SV40- or CMV-promoter-driven CAT (chloramphenicol-acetyl-transferase)expression plasmids using DOTAP as the liposome carrier. The level of CAT expression was shown to correlate directly with the amount of transfected DNA and could be measured by a CAT-enzyme-linked immunosorbent assay. The results of gene transfer by liposome-DNA complexes obtained for all cell lines showed a dose-dependent efficacy correlating to the amount of DOTAP employed. The data demonstrate the successful in vitro transfection of epithelial cell lines with DNA, suggesting its usefulness as a new tool for head and neck cancer therapy in vivo.

L8 ANSWER 4 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9

ACCESSION NUMBER: 1996:24809 BIOSIS
DOCUMENT NUMBER: PREV199698596944
TITLE: Genetic radiotherapy overcomes tumor resistance to cytotoxic agents.
AUTHOR(S): Seung, Lisa P.; Mauceri, Helena J.; Beckett, Michael A.; Hallahan, Dennis E.; Hellman, Samuel; Weichselbaum, Ralph R. (1)
CORPORATE SOURCE: (1) Dep. Radiat. Cell. Oncol., Univ. Chicago Med. Cent., 5841 South Maryland Ave., Box 442, Chicago, IL 60637 USA
SOURCE: Cancer Research, (1995) Vol. 55, No. 23, pp. 5561-5565.
ISSN: 0008-5472.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We report that **radiation** enhances gene therapy of a radioresistant tumor by upregulating the induction of a chimeric gene encoding a radiosensitizing protein, tumor necrosis factor alpha (TNF-alpha). We ligated the **radiation**-inducible CArG elements of the **radiation**-inducible Egr-1 promoter/enhancer region upstream to the transcriptional start site of the human TNF cDNA (pE425-TNF). This construct was transfected using **cationic liposomes**

into the variant murine fibrosarcoma cell line, P4L. The P4L cell line was both radioresistant (D-0 = 188) and resistant to TNF. After a single intratumoral injection of 10 mu-g of pE425-TNF in **cationic liposomes** and two 20-Gy doses of irradiation, mean tumor volumes were significantly reduced in P4L tumors as compared to those receiving either pE425-TNF in liposomes or **radiation** alone (P = 0.01). TNF protein in P4L tumors was induced by **radiation** as high as 29 times control levels and remained detectable for 14 days. Our data indicate that combined gene therapy using liposomes, together with ionizing **radiation** to locally activate the induction of a radiosensitizing protein, is successful at overcoming resistance to both TNF and **radiation**.

L8 ANSWER 5 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:415075 BIOSIS
DOCUMENT NUMBER: PREV200000415075
TITLE: Ultrasound enhancement of liposome-mediated cell transfection is caused by cavitation effects.
AUTHOR(S): Koch, Sandra; Pohl, Peter; Cobet, Ulrich; Rainov, Nikolai G. (1)
CORPORATE SOURCE: (1) Department of Neurosurgery, Martin-Luther-University Halle, Magdeburger Str. 16, D-06097, Halle Germany
SOURCE: Ultrasound in Medicine and Biology, (June, 2000) Vol. 26, No. 5, pp. 897-903. print.
ISSN: 0301-5629.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Cationic liposomes** (CL) are widely used vectors for gene transfer. Recently, ultrasound (US) was reported to enhance liposome-mediated gene transfer to eucaryotic cells in culture. The present study was aimed at studying the effects of 2-MHz pulsed Doppler US on malignant brain tumor cells transfection by **cationic liposome**/plasmid-DNA complexes (lipoplexes). **Cationic liposomes** consisting of DOSPA/DOPE were complexed with a plasmid carrying the cDNA encoding green autofluorescent protein (EGFP). Rodent (9L) and canine (J3T) glioma cells were exposed to pulsed US in the presence of EGFP-lipoplexes. A diagnostic transcranial Doppler device (MultiDop L) was used for insonation for 30, 60, and 90 s at 2 MHz/0.5 W/cm2. To eliminate US reflection and cavitation, a custom-made absorption chamber was designed, where US is applied through a water tank before interacting with the cells and is fully absorbed after passing through the cell layer. Expression of the marker gene EGFP was quantified by FACS analysis and intravital fluorescent microscopy. Cell viability was accessed by Trypan Blue staining. US treatment of tumor cells on microplates for 60 s yielded a significant increase in transfection rates without damaging the cells, but 90-s treatment killed most of the cells. In the absorption chamber, no significant effects of US on transfection were noted. Additional experiments employed US contrast agent (Levovist(R), Schering) which was able to significantly increase tumor cell transfection rate by enhancing cavitation effects, and also severely damaged most cells when applied at a concentration of 200 mg/mL. In conclusion, our results support the assumption that US effects on lipoplex transfection rates in brain tumor cells in culture are mediated by cavitation effects.

L8 ANSWER 6 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:514295 BIOSIS
DOCUMENT NUMBER: PREV200000514295
TITLE: Effect of low frequency, low amplitude magnetic fields on the permeability of **cationic liposomes** entrapping carbonic anhydrase: II. No evidence for surface enzyme involvement.
AUTHOR(S): Ramundo-Orlando, Alfonsina; Mattia, Francesca; Palombo,

Alessandro; D'Inzeo, Guglielmo (1)
CORPORATE SOURCE: (1) Department of Electronic Engineering, University of Rome "La Sapienza", Via Eudossiana, 18, 00184, Rome Italy
SOURCE: Bioelectromagnetics, (October, 2000) Vol. 21, No. 7, pp. 499-507. print.
ISSN: 0197-8462.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Observations recently reported by our group indicate that combined 7 Hz sinusoidal (Bacpeak = 50 muT) and parallel static (Bdc = 50 muT) magnetic fields can induce a significant increase in diffusion rate of substrate across carbonic anhydrase (CA)-loaded liposomes (DPPC:Chol:SA). A direct involvement of charges of stearylamine (SA) on the lipid membrane surface was also demonstrated. Kinetic studies showed that CA was mainly entrapped in liposomes at 5:3:2 molar ratio, although a small amount (17%) of enzyme was also located on the external surface of these **cationic liposomes**. In this paper we report steady state kinetic studies on this latter CA after ELF-EMFs exposure. No difference in the apparent Km between exposed and sham samples was observed. On the contrary the apparent Vmax was increased by approximately a factor of 2 after field exposure. In spite of the proteolytic digestion of this external CA, a significant increase of enzymatic activity, as a function of increase in the diffusion rate of substrate across the lipid bilayer, was observed in the exposed samples. Based on these results, a conformational change induced by the field on the CA located on the external surface of 5:3:2 liposomes is excluded as an explanation for our previous observations, supporting the primary role of bilayer SA in the interaction with ELF. A model of ELF interaction, based on the Larmor precession theory, explaining the physical phenomenon induced on the dipole of SA has been developed.

L8 ANSWER 7 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:514294 BIOSIS
DOCUMENT NUMBER: PREV200000514294
TITLE: Effect of low frequency, low amplitude magnetic fields on the permeability of **cationic liposomes** entrapping carbonic anhydrase: I. Evidence for charged lipid involvement.
AUTHOR(S): Ramundo-Orlando, Alfonsina; Morbiducci, Umberto; Mossa, Giuseppe; D'Inzeo, Guglielmo (1)
CORPORATE SOURCE: (1) Department of Electronic Engineering, University of Rome "La Sapienza", Via Eudossiana, 18, 00184, Rome Italy
SOURCE: Bioelectromagnetics, (October, 2000) Vol. 21, No. 7, pp. 491-498. print.
ISSN: 0197-8462.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The influence of low frequency (4-16 Hz), low amplitude (25-75 muT) magnetic fields on the diffusion processes in enzyme-loaded unilamellar liposomes as bioreactors was studied. **Cationic liposomes** containing dipalmitoylphosphatidylcholine, cholesterol, and charged lipid stearylamine (SA) at different molar ratios (6:3:1 or 5:3:2) were used. Previous kinetic experiments showed a very low self-diffusion rate of the substrate p-nitrophenyl acetate (p-NPA) across intact liposome bilayer. After 60 min of exposure to 7 Hz sinusoidal (50 muT peak) and parallel static (50 muT) magnetic fields the enzyme activity, as a function of increased diffusion rate of p-NPA, rose from 17+-3% to 80+-9% ($P < .0005$, $n = 15$) in the 5:3:2 liposomes. This effect was dependent on the SA concentration in the liposomes. Only the presence of combined sinusoidal (AC) and static (DC) magnetic fields affected the p-NPA diffusion rates. No enzyme leakage was observed. Such studies suggest a plausible link between the action of extremely low frequency magnetic field on charged

lipids and a change of membrane permeability.

L8 ANSWER 8 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:281661 BIOSIS
DOCUMENT NUMBER: PREV199900281661
TITLE: Antisense raf oligodeoxyribonucleotide is a radiosensitizer in vivo.
AUTHOR(S): Gokhale, Prafulla C.; McRae, Donald; Monia, Brett P.; Bagg, Adam; Rahman, Aquilur; Dritschilo, Anatoly; Kasid, Usha (1)
CORPORATE SOURCE: (1) Georgetown University Medical Center, 3970 Reservoir Road, NW, E208, Research Building, Washington, DC, 20007 USA
SOURCE: Antisense & Nucleic Acid Drug Development, (April, 1999) Vol. 9, No. 2, pp. 191-201.
ISSN: 1087-2906.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Raf-1, a cytosolic protein serine/threonine kinase, plays important roles in cell growth, proliferation, transformation, and cell survival. The aim of the present study was to evaluate the radiotherapeutic efficacy of a fully phosphorothioated and well-characterized antisense raf oligodeoxyribonucleotide (ODN) corresponding to the 3'-untranslated region of human c-raf-1 mRNA (ISIS 5132/5132). Using our recently developed liposome encapsulation of ODN approach, we first compared the pharmacokinetic parameters of a liposomal formulation of 5132 (LE-5132) and 5132. The peak plasma concentrations 5 minutes after ODN administrations (30 mg/kg i.v.) were 28.5 mug/ml and 13.5 mug/ml for LE-5132 and 5132, respectively. The decrease in plasma concentration of LE-5132 and 5132 followed a biexponential pattern, with initial distribution half-lives (t1/2alpha) of 34.8 minutes and 21.6 minutes, respectively. The terminal half-lives (t1/2beta) with LE-5132 and 5132 were 14.5 hours and 4.3 hours,respectively. The area under the plasma concentration-time curve (AUC) was 5.8 times higher with LE-5132 than with 5132. Significantly higher intact ODN levels could be measured in most organs within 48 hours of administration of LE-5132 compared with 5132 (liver 18.4-fold, spleen, 31-fold, heart 3-fold, lungs 1.5-fold). In kidneys, the level was lower with LE-5132 (0.77-fold). LE-5132 composition, unlike 5132, did not affect clotting time in vitro. Significant decline in the level of Raf-1 protein was observed in vitro in relatively radioresistant human laryngeal squamous cell carcinoma cells (SQ-20B) treated with LE-5132 compared with SQ-20B cells treated with equimolar concentration of 5132 or liposome-encapsulated mismatched 5132 (0.5 muM LE-5132, 71.3% +- 22.5%; 1.0 muM LE-5132, 79.6% +- 16.7%). In addition, LE-5132 appeared to be a more potent antitumor compound than 5132 ($p < 0.001$). These data established the suitability of LE-5132 for in vivo radio-therapeutic efficacy studies. Intravenous administration of LE-5132 into SQ-20B tumor-bearing athymic mice inhibited Raf-1 expression in tumor tissue compared with blank liposome-treated or untreated control groups. LE-5132 or ionizing **radiation** (IR) treatment alone caused significant but transient inhibition of SQ-20B tumor growth but not tumor regression. Remarkably, a combination of LE-5132 and IR treatments led to significant and sustained tumor regression for at least 27 days after the last treatment ($p < 0.001$). Histopathologic examination of tumor samples revealed a significant proportion of cells containing fragmented chromatin in the LE-5132 + IR treatment group as compared with single agent and untreated control groups. These in vivo data support the notion that Raf-1 has proliferative and survival functions and advance the scientific and technologic bases for the use of antisense raf ODN in the management of radioresistant malignancies.

L8 ANSWER 9 OF 26 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001443723 MEDLINE
DOCUMENT NUMBER: 21382303 PubMed ID: 11489488

TITLE: Tumor-targeted p53-gene therapy enhances the efficacy of conventional chemo/radiotherapy.
AUTHOR: Xu L; Pirollo K F; Chang E H
CORPORATE SOURCE: Department of Oncology, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC, USA.
CONTRACT NUMBER: R01 CA45158 (NCI)
SOURCE: JOURNAL OF CONTROLLED RELEASE, (2001 Jul 6) 74 (1-3) 115-28. Ref: 55
Journal code: 8607908. ISSN: 0168-3659.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20020121
Entered Medline: 20011204

AB A long-standing goal in gene therapy for cancer is a stable, low toxic, systemic gene delivery system that selectively targets tumor cells, including metastatic disease. Progress has been made toward developing non-viral, pharmaceutical formulations of genes for in vivo human therapy, particularly **cationic liposome**-mediated gene transfer systems. Ligand-directed tumor targeting of **cationic liposome**-DNA complexes (lipoplexes) is showing promise for targeted gene delivery and systemic gene therapy. Lipoplexes directed by ligands such as folate, transferrin or anti-transferrin receptor scFv, showed tumor-targeted gene delivery and expression in human breast, prostate, head and neck cancers. The two elements, ligand/receptor and liposome composition, work together to realize the goal of functional tumor targeting of gene therapeutics. The tumor suppressor gene, p53, has been shown to be involved in the control of DNA damage-induced apoptosis. Loss or malfunction of this p53-mediated apoptotic pathway has been proposed as one mechanism by which tumors become resistant to chemotherapy or **radiation**. The systemically delivered ligand-liposome-p53 gene therapeutics resulted in efficient expression of functional wild-type p53, sensitizing the tumors to chemotherapy and radiotherapy. This is a novel strategy combining current molecular medicine with conventional chemotherapy and radiotherapy for the treatment of cancer. The systemic delivery of normal tumor suppressor gene p53 by a non-viral, tumor-targeted delivery system as a new therapeutic intervention has the potential to critically impact the clinical management of cancer.

L8 ANSWER 10 OF 26 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001636319 MEDLINE
DOCUMENT NUMBER: 21546327 PubMed ID: 11690554
TITLE: Improvements in gene therapy technologies.
AUTHOR: Kaneda Y
CORPORATE SOURCE: Division of Gene Therapy Science, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan.. kaneday@gts.med.osaka-u.ac.jp
SOURCE: MOLECULAR UROLOGY, (2001 Summer) 5 (2) 85-9. Ref: 29
Journal code: 9709255. ISSN: 1091-5362.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011107
Last Updated on STN: 20020216
Entered Medline: 20020215

AB We have combined hemagglutinating virus of Japan (HVJ; Sendai virus) with liposomes for efficient in vitro and in vivo fusion-mediated gene delivery. The HVJ-liposome was a highly efficient vehicle for the introduction of oligonucleotides into cells in vivo as well as for the transfer of genes <100 kbp without damaging cells. By coupling the Epstein-Barr (EB) virus replicon apparatus with HVJ-liposomes (virosomes), transgene expression was sustained in vitro and in vivo. When we added cationic lipids, the HVJ-**cationic liposomes** increased gene delivery 100 to 800 times in vitro compared with the conventional anionic virosomes and were also more useful for gene expression in restricted areas of organs and for gene therapy of disseminated cancers. We further discovered that the use of anionic virosomes with a virus-mimicking lipid composition (artificial viral envelope; AVE type) increased transfection efficiency approximately 10 fold in vivo, especially in the heart, liver, kidney, and muscle. Most animal organs were found to be suitable targets for the fusigenic virosomes, and numerous gene therapy strategies using this system were successful in animals. The combination of suicide gene therapy with **radiation** was very effective for killing hepatomas in a mouse model. Arteriosclerosis obliterans in animal models was cured by the transfer of hepatocyte growth factor.

L8 ANSWER 11 OF 26 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97207028 MEDLINE
DOCUMENT NUMBER: 97207028 PubMed ID: 9054521
TITLE: Transferrin-liposome-mediated p53 sensitization of squamous cell carcinoma of the head and neck to **radiation** in vitro.
AUTHOR: Xu L; Pirollo K F; Chang E H
CORPORATE SOURCE: Department of Surgery, Division of Otolaryngology, Stanford University Medical Center, CA 94305-5328, USA.
CONTRACT NUMBER: R01 CA45158 (NCI)
SOURCE: HUMAN GENE THERAPY, (1997 Mar 1) 8 (4) 467-75. Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970514
Last Updated on STN: 19970514
Entered Medline: 19970508

AB Wild-type (wt) p53 DNA was transfected into the radioresistant human cell line JSQ-3, established from a squamous cell carcinoma of the head and neck (SCCHN), using a transferrin-liposome system, and the ability of the introduced wt p53 to sensitize the transfected JSQ-3 cells to ionizing **radiation** was examined. Transferrin increased the in vitro transfection efficiency of **cationic liposomes** up to 70-80% in JSQ-3 cells, representing a 6- to 10-fold increase over liposome transfection alone. The exogenous wt p53 was expressed at high levels in transferrin-liposome-DNA-transfected cells and resulted in the reversion of the radioresistant phenotype of the JSQ-3 cells in a DNA dose-dependent manner. The D10 values were reduced from 6.36 +/- 0.54 Gy to 4.13 +/- 0.06 Gy, a value in the radiosensitive range. In vivo, the intratumoral injection of the transferrin-liposome system resulted in a higher number of transfected tumor cells in the JSQ-3 induced nude mouse xenografts when compared with transfection by liposome alone. The results indicate that the combination of p53 replacement gene transduction, mediated by the relatively safe transferrin-liposome system, and conventional ionizing **radiation** may provide a more effective treatment for head and neck cancer.

L8 ANSWER 12 OF 26 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999034993 MEDLINE

DOCUMENT NUMBER: 99034993 PubMed ID: 9816091
TITLE: Gene modification of primary tumor cells for active immunotherapy of human breast and ovarian cancer.
AUTHOR: Philip R; Clary B; Brunette E; Kilinski L; Murugesh D; Sorich M; Yau J; Lebkowski J; Lyerly H K; Philip M
CORPORATE SOURCE: Applied Immune Sciences, Inc., Santa Clara, California 95054-1114, USA.
SOURCE: CLINICAL CANCER RESEARCH, (1996 Jan) 2 (1) 59-68. Journal code: 9502500. ISSN: 1078-0432.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990223
Last Updated on STN: 19990223
Entered Medline: 19990210

AB We have previously shown that **cationic liposomes** facilitate adeno-associated virus (AAV) plasmid transfections of primary and cultured cell types. To test the clinical feasibility of using genetically modified tumor vaccines for the treatment of breast and ovarian cancers, we have constructed an expression plasmid pMP6IL2 and investigated the use of liposome-mediated gene delivery into primary, uncultured human breast and ovarian tumor cells to produce interleukin 2 (IL-2)-secreting tumor cells. We have demonstrated significant levels of IL-2 expression in tumor cell lines and primary breast and ovarian tumor cells using this AAV-based expression plasmid complexed to **cationic liposomes**. Transfections with the non-AAV plasmid containing the identical expression cassette as the AAV plasmid induced IL-2 expression in the tumor cell line but failed to produce IL-2 in primary tumor cells. Significant levels of IL-2 were induced with the AAV plasmid regardless of liposome compositions used for transfection. The transfected breast cell line and primary tumor cells were able to express the transgene product for up to 28 days after lethal **radiation**. The transfection efficiency was comparable for both the tumor cell line and primary tumor cells and ranged from 20 to 50% for both cell types as assessed by intracellular IL-2 staining. Although the primary tumor cell preparations consist of mixed population of cells, at least 40% of the tumor cells expressed the transgene as assessed by immunostaining for IL-2. The ability to efficiently express transgenes in freshly isolated, nondividing tumor cells may potentiate active immunotherapy strategies for gene-based cancer treatment.

L8 ANSWER 13 OF 26 MEDLINE
ACCESSION NUMBER: 2002408860 MEDLINE
DOCUMENT NUMBER: 22152947 PubMed ID: 12030844
TITLE: Interference of poly(ethylene glycol)-lipid analogues with cationic-lipid-mediated delivery of oligonucleotides; role of lipid exchangeability and non-lamellar transitions.
AUTHOR: Shi Fuxin; Wasungu Luc; Nomden Anita; Stuart Marc C A; Polushkin Evgeny; Engberts Jan B F N; Hoekstra Dick
CORPORATE SOURCE: Department of Membrane Cell Biology, Faculty of Medical Sciences, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.
SOURCE: BIOCHEMICAL JOURNAL, (2002 Aug 15) 366 (Pt 1) 333-41. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 20020807
Last Updated on STN: 20021010
Entered Medline: 20021008

AB **Cationic liposomes** are applied to transfer oligonucleotides (ODNs) into cells to regulate gene expression for gene therapeutic or cell biological purposes. In vivo, poly(ethylene glycol) (PEG)-lipid derivatives are employed to stabilize and prolong the circulation lifetime of nucleic acid-containing particles, and to improve targeting strategies. In this study, we have studied the effects of PEG-lipid analogues, i.e. PEG coupled to either phosphatidylethanolamine (PE) or ceramide, on cationic-lipid-DNA complex ('lipoplex') assembly and the mechanism of cationic-lipid-mediated delivery of ODNs in vitro. Inclusion of 10 mol% PEG-PE in ODN lipoplexes inhibited their internalization in Chinese hamster ovary cells by more than 70%. The intracellular fraction remained entrapped in the endosomal/lysosomal pathway, and no release of ODNs was apparent. Similar observations were made for complexes prepared from liposomes that contained PEG-ceramides. Interestingly, delivery resumed when lipoplexes had been externally coated with PEG-ceramides. In this case, the kinetics of delivery were dependent on the length of the ceramide acyl chain, consistent with a requirement for the PEG-lipid to dissociate from the complex. Moreover, although the chemical nature of the PEG-ceramides distinctly affected the net internalization of the complexes, impediment of delivery was largely related to an inhibitory effect of the PEG-lipid on the release of ODNs from the endosomal compartment. Cryo-electron microscopy and small-angle X-ray scattering revealed that the PEG-lipids stabilize the lamellar phase of the lipoplexes, while their acyl-chain-length-dependent transfer from the complex enables adaptation of the hexagonal phase. Within the endosomal compartment, this transition appears to be instrumental in causing the dissociation and cytosolic release of the ODNs for their nuclear homing.

L8 ANSWER 14 OF 26 MEDLINE

ACCESSION NUMBER:	2001423294 MEDLINE
DOCUMENT NUMBER:	21225062 PubMed ID: 11325732
TITLE:	Spontaneous entrapment of polynucleotides upon electrostatic interaction with ethanol-destabilized **cationic liposomes**.
AUTHOR:	Maurer N; Wong K F; Stark H; Louie L; McIntosh D; Wong T; Scherrer P; Semple S C; Cullis P R
CORPORATE SOURCE:	Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3.. nmaurer@interchange.ubc.ca
SOURCE:	BIOPHYSICAL JOURNAL, (2001 May) 80 (5) 2310-26. Journal code: 0370626. ISSN: 0006-3495.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	200107
ENTRY DATE:	Entered STN: 20010730 Last Updated on STN: 20010730 Entered Medline: 20010726

AB This study describes the effect of ethanol and the presence of poly(ethylene) glycol (PEG) lipids on the interaction of nucleotide-based polyelectrolytes with **cationic liposomes**. It is shown that preformed large unilamellar vesicles (LUVs) containing a cationic lipid and a PEG coating can be induced to entrap polynucleotides such as antisense oligonucleotides and plasmid DNA in the presence of ethanol. The interaction of the **cationic liposomes** with the polynucleotides leads to the formation of multilamellar liposomes ranging in size from 70 to 120 nm, only slightly bigger than the parent LUVs from which they originated. The degree of lamellarity as well as the size and polydispersity of the liposomes formed increases with increasing polynucleotide-to-lipid ratio. A direct correlation between the entrapment efficiency and the membrane-destabilizing effect of ethanol was observed. Although the morphology of the liposomes is still preserved at the ethanol

concentrations used for entrapment (25-40%, v/v), entrapped low-molecular-weight solutes leak rapidly. In addition, lipids can flip-flop across the membrane and exchange rapidly between liposomes. Furthermore, there are indications that the interaction of the polynucleotides with the **cationic liposomes** in ethanol leads to formation of polynucleotide-cationic lipid domains, which act as adhesion points between liposomes. It is suggested that the spreading of this contact area leads to expulsion of PEG-ceramide and triggers processes that result in the formation of multilamellar systems with internalized polynucleotides. The high entrapment efficiencies achieved at high polyelectrolyte-to-lipid ratios and the small size and neutral character of these novel liposomal systems are of utility for liposomal delivery of macromolecular drugs.

L8 ANSWER 15 OF 26 MEDLINE
ACCESSION NUMBER: 2001167455 MEDLINE
DOCUMENT NUMBER: 21165826 PubMed ID: 11269338
TITLE: Atomic force microscopy imaging of DNA-**cationic liposome** complexes optimised for gene transfection into neuronal cells.
AUTHOR: Wangerek L A; Dahl H H; Senden T J; Carlin J B; Jans D A; Dunstan D E; Ioannou P A; Williamson R; Forrest S M
CORPORATE SOURCE: Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia.
SOURCE: JOURNAL OF GENE MEDICINE, (2001 Jan-Feb) 3 (1) 72-81. Journal code: 9815764. ISSN: 1099-498X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

AB BACKGROUND: **Cationic liposomes** represent an important gene delivery system due to their low immunogenicity, but are relatively inefficient, with optimisation of DNA-liposome complexes (lipoplexes) for transfection necessary for each cell type of interest. There have been few studies examining optimisation in neuronal cell types or determining how the structure of lipoplexes affects transfection efficiency. METHODS: Four commercially available **cationic liposome** formulations were used to optimise transfection efficiency in neuronal cells. The DNA to liposome ratio and the amount of DNA used in transfections were varied. Transfection efficiency was determined by the percentage of cells positive for the micro-galactosidase reporter gene product. The structure of lipoplexes was studied using atomic force microscopy. Lipoplexes were characterised further using dynamic light scattering to determine size and fluorescence techniques to show DNA compaction. RESULTS: Optimal transfection conditions were found to differ between immortalised cell lines and primary cells. High transfection efficiencies in immortalised cell lines were achieved predominantly with multivalent **cationic liposomes** while primary neuronal cells showed optimal transfection efficiency with monovalent **cationic liposomes**. The structure of lipoplexes was observed with atomic force microscopy and showed globular complexes for multivalent **cationic liposomes**, while monovalent liposomes gave less compact structures. In support of this finding, high levels of DNA compaction with multivalent liposomes were observed using fluorescence quenching measurements for all DNA to liposome ratios tested. One monovalent liposome showed increasing levels of compaction with increasing liposome amount. Dynamic light scattering showed little change in complex size when the different lipoplexes were studied. CONCLUSIONS: Optimisation of transfection efficiency was different for cell lines and primary neurons. Immortalised cells showed optimal transfection with multivalent liposomes

while primary neurons showed optimal transfection with monovalent liposomes. The charge ratio of the monovalent liposome was below one, suggesting a different mechanism of lipoplex binding and uptake in primary neurons. The structure of lipoplexes, as

L8 ANSWER 16 OF 26 MEDLINE
ACCESSION NUMBER: 1998119678 MEDLINE
DOCUMENT NUMBER: 98119678 PubMed ID: 9459590
TITLE: Electrostatic and structural properties of complexes involving plasmid DNA and cationic lipids commonly used for gene delivery.
AUTHOR: Zuidam N J; Barenholz Y
CORPORATE SOURCE: Department of Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1998 Jan 5) 1368 (1) 115-28.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 19980226
Entered Medline: 19980218

AB The present study is aimed to characterize the interactions between plasmid DNA and cationic, large unilamellar vesicles, 110+/-20nm in size, composed of lipids commonly used for transfections including DOTAP/DOPE (mole ratio 1/1), DOTAP/DOPC (mole ratio 1/1), 100% DOTAP, or DC-CHOL/DOPE (mole ratio 1/1). [Abbreviations:DOTAP, N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; DC-CHOL, 3 beta-[N-(N',N'-dimethylaminoethane)carbamoyl] cholesterol]. A novel approach of combining Gouy-Chapman calculations and fluorescence measurements of the pH at the surface of lipid assemblies by the fluorophore 4-heptadecyl-7-hydroxycoumarin showed that electrostatic parameters played a key role in the instantaneous formation of the DNA-lipid complexes upon addition of different amounts of plasmid DNA to **cationic liposomes** in 20 mM Hepes buffer (pH 7.4). Addition of large amounts of plasmid DNA leads to neutralization of 60% of the protonated DC-CHOL in DC-CHOL/DOPE (1/1) assemblies and 80% of the DOTAP in lipid assemblies. The characterization of these electrostatic parameters of the complexes suggests better and closer surrounding of plasmid DNA by lipids when DOPE is present. Time-dependent static light-scattering measurements monitored the formation of complexes and also showed that these complexes were highly unstable with respect to size at DNA/cationic lipid molar ratios between 0.2 and 0.8.

L8 ANSWER 17 OF 26 MEDLINE
ACCESSION NUMBER: 97165761 MEDLINE
DOCUMENT NUMBER: 97165761 PubMed ID: 9012343
TITLE: Structure of DNA-**cationic liposome** complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes.
COMMENT: Comment in: Science. 1997 Feb 7;275(5301):791-2
AUTHOR: Radler J O; Koltover I; Salditt T; Safinya C R
CORPORATE SOURCE: Materials Department, University of California, Santa Barbara, CA 93106, USA.
SOURCE: SCIENCE, (1997 Feb 7) 275 (5301) 810-4.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19980206
Entered Medline: 19970224

AB **Cationic liposomes** complexed with DNA (CL-DNA) are promising synthetically based nonviral carriers of DNA vectors for gene therapy. The solution structure of CL-DNA complexes was probed on length scales from subnanometer to micrometer by synchrotron x-ray diffraction and optical microscopy. The addition of either linear lambda-phage or plasmid DNA to CLs resulted in an unexpected topological transition from liposomes to optically birefringent liquid-crystalline condensed globules. X-ray diffraction of the globules revealed a novel multilamellar structure with alternating lipid bilayer and DNA monolayers. The lambda-DNA chains form a one-dimensional lattice with distinct interhelical packing regimes. Remarkably, in the isoelectric point regime, the lambda-DNA interaxial spacing expands between 24.5 and 57.1 angstroms upon lipid dilution and is indicative of a long-range electrostatic-induced repulsion that is possibly enhanced by chain undulations.

L8 ANSWER 18 OF 26 MEDLINE
ACCESSION NUMBER: 1998051631 MEDLINE
DOCUMENT NUMBER: 98051631 PubMed ID: 9390192
TITLE: Maintenance of transfection rates and physical characterization of lipid/DNA complexes after freeze-drying and rehydration.
AUTHOR: Anchordoquy T J; Carpenter J F; Kroll D J
CORPORATE SOURCE: Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver 80262, USA.
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1997 Dec 1) 348 (1) 199-206.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980116
Last Updated on STN: 19980116
Entered Medline: 19971224

AB It is well established that **cationic liposomes** form complexes with DNA and effectively transfect cells in vivo and ex vivo. Lipid/DNA complexes have proven safe and nonimmunogenic in clinical trials; however, they are known to aggregate readily in liquid formulations. This physical instability requires clinicians to prepare lipid/DNA complexes immediately prior to injection. In order to eliminate problems associated with this temporal requirement, we investigated the feasibility of preserving complexes as a dried preparation that could be tested, stored, and rehydrated as needed. To this end, our study evaluated the ability of different stabilizers to preserve transfection rates of complexes during acute freeze-drying stress. Our data show that complexes lyophilized in 0.5 M sucrose or trehalose possessed transfection rates similar to those of fresh preparations. In addition, dried complexes that exhibited full transfection activity upon rehydration had sizes comparable to nonlyophilized controls. Our work demonstrates that lipid/DNA complexes can be stabilized as dried powders that offer significant advantages over current liquid formulations. Furthermore, the correlation of transfection rates with maintenance of complex diameter suggests that size plays a critical role in lipid-based DNA delivery.

L8 ANSWER 19 OF 26 MEDLINE
ACCESSION NUMBER: 96439887 MEDLINE
DOCUMENT NUMBER: 96439887 PubMed ID: 8842198
TITLE: The role of helper lipids in **cationic**

liposome-mediated gene transfer.
AUTHOR: Hui S W; Langner M; Zhao Y L; Ross P; Hurley E; Chan K
CORPORATE SOURCE: Biophysics Department, Roswell Park Cancer Institute, Buffalo, New York 14263, USA. roswhui@ubvms.cc buffalo.edu.
CONTRACT NUMBER: GM30969 (NIGMS)
SOURCE: BIOPHYSICAL JOURNAL, (1996 Aug) 71 (2) 590-9.
Journal code: 0370626. ISSN: 0006-3495.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970109

AB In the procedure for **cationic liposome**-mediated transfection, the cationic lipid is usually mixed with a "helper lipid" to increase its transfection potency. The importance of helper lipids, including dioleoylphosphatidylcholine (DOPC) and phosphatidylethanolamine (dioleoyl PE), DO was examined. Freeze-fracture electron microscopy of DNA:cationic complexes containing the pSV-beta-GAL plasmid DNA, the cationic lipid dioleoyl trimethylammonium propane, and these helper lipids showed that the most efficient mixtures were aggregates of ensheathed DNA and fused liposomes. PE-containing complexes aggregated rapidly when added to culture media containing polyanions, whereas PC-containing complexes did not. However, more granules of PC-containing complexes were formed on cell surfaces after the complexes were added to Chinese hamster ovary (CHO) cells in transfection media. Pronase treatment inhibited transfection, whereas dilute poly-L-lysine enhanced transfection, indicating that the attachment of DNA:liposome complexes to cell surfaces was mediated by electrostatic interaction. Fluorescence spectroscopy studies confirmed that more PC-containing complexes than PE-containing complexes were associated with CHO cells, and that more PC-containing complexes were located in a low pH environment (likely to be within endosomes) with time. Cytochalasin-B had a stronger inhibitory effect on PC-containing liposome-mediated than on PE-containing liposome-mediated transfection. Confocal microscopic recording of the fluorescently label lipid and DNA uptake process indicated that many granules of DNA: **cationic liposome** complexes were internalized as a whole, whereas some DNA aggregates were left out on the cell surfaces after liposomes of the complexes fused with the plasma membranes. For CHO cells, endocytosis seems to be the main uptake pathway of DNA: **cationic liposome** complexes. More PC-containing granules than PE-containing granules were formed on cell surfaces by cytoskeleton-directed membrane motion, after their respective DNA:liposome complexes attached to cell surfaces by electrostatic means. Formation of granules on the cell surface facilitated and/or triggered endocytosis. Fusion between **cationic liposomes** and the cell membrane played a secondary role in determining transfection efficiency.

L8 ANSWER 20 OF 26 MEDLINE
ACCESSION NUMBER: 89025936 MEDLINE
DOCUMENT NUMBER: 89025936 PubMed ID: 3178866
TITLE: Controlled human RBC modifications affecting the binding of **cationic liposomes**.
AUTHOR: Di Giulio A; Oratore A; Tozzi-Ciancarelli M G; Crifo' C; Finazzi-Agro' A
CORPORATE SOURCE: Dept. of Biomedical Sciences and Technologies, University of L'Aquila, Roma, Italy.
SOURCE: BIOCHEMISTRY INTERNATIONAL, (1988 Jun) 16 (6) 999-1007.
Journal code: 8100311. ISSN: 0158-5231.
PUB. COUNTRY: Australia
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19881121

AB **Cationic liposomes** were prepared either by sonication or by detergent dialysis and used to deliver the antioxidative enzyme glutathione peroxidase into human erythrocytes in vitro. The enrichment ability of these two preparations was similar, amounting to about 30% of the control cells. The lysis of enzyme-treated erythrocytes induced by photoirradiation in the presence of PPIX was compared with that of cells incubated with empty liposomes. Erythrocytes enriched with GPX appear to be more resistant toward photohemolysis. Pre-treatment of cells with neuraminidase or proteinase K suggests that: a) sialic acid seems to be essential for the cell-liposome fusion process, no enrichment being found with the neuraminidase-treated cells; b) hydrolysis of the outer membrane proteins leads to an increased fragility with respect to controls even in GPX-enriched cells. These results were confirmed by extrinsic fluorescence polarization experiments, using isolated erythrocyte membranes and specific fluorescent probes.

L8 ANSWER 21 OF 26 MEDLINE
ACCESSION NUMBER: 84058263 MEDLINE
DOCUMENT NUMBER: 84058263 PubMed ID: 6227497
TITLE: Differential sensitivity to photohemolysis of erythrocytes enriched with some liposome-carried substances.
AUTHOR: Finazzi-Agro A; Aquilio E; Crifo C
SOURCE: EXPERIENTIA, (1983 Nov 15) 39 (11) 1298-9.
Journal code: 0376547. ISSN: 0014-4754.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198401
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19840127

AB The sensitivity of human erythrocytes to photohemolysis sensitized by addition of protoporphyrin IX can be selectively affected by their enrichment with substances carried by **cationic liposomes** . In particular the enrichment which superoxide dismutase is accompanied by a copper-related greater sensitivity toward photohemolysis, as observed in the Down's syndrome (mongolism). Instead it is possible to protect the erythrocytes against the phototoxic effect of protoporphyrin by enrichment with small amounts of beta-carotene.

L8 ANSWER 22 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 1999:586523 CAPLUS
DOCUMENT NUMBER: 131:254392
TITLE: Systemic p53 gene therapy in combination with **radiation** results in human tumor regression
AUTHOR(S): Xu, L.; Pirollo, K. F.; Rait, A.; Murray, A. L.; Chang, E. H.
CORPORATE SOURCE: Department of Otolaryngology Head and Neck Surgery, Washington, DC, USA
SOURCE: Tumor Targeting (1999), 4(2), 92-104
CODEN: TUTAF9; ISSN: 1351-8488
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A long-standing goal in gene therapy for cancer is a systemic delivery system that selectively targets tumor cells including metastases. We optimized a folate contg. **cationic liposome** system for the systemic delivery of wtp53 to squamous cell carcinoma of the head and

neck. The folate ligand, which serves to target the complex to tumor cells, increased the transfection efficiency by facilitating transient gene transfection. This system was demonstrated to be exceedingly tumor-selective in that normal tissues, including the highly proliferative gut and bone marrow, were not transfected. The systemic delivery by this method of wild-type p53 to established mouse xenografts markedly sensitized these human tumors to radiotherapy. This combination of systemic p53 gene therapy and conventional radiotherapy resulted in complete tumor regression and inhibition of their recurrence long-term. Similar results were also demonstrated with another model system, prostate cancer cell line DU145. This addn. of a mol. component could provide an improved therapeutic approach for cancers of the head and neck and other forms of cancer as well.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 23 OF 26 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:319207 CAPLUS
DOCUMENT NUMBER: 133:187500
TITLE: Targeted p53 gene therapy-mediated radiosensitization and chemosensitization
AUTHOR(S): Chang, Esther H.; Xu, Liang; Pirollo, Kathleen F.
CORPORATE SOURCE: Department of Otolaryngology, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC, USA
SOURCE: Signaling Networks and Cell Cycle Control (2000), 519-536. Editor(s): Gutkind, J. Silvio. Humana Press Inc.: Totowa, N. J.
CODEN: 68YVA9
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English

AB A review with 139 refs., describing the nonviral delivery of a functional tumor suppressor gene (encoding p53) to cancer cells. Delivery systems for gene therapy, the p53 gene and cancer, tumor-targeted **cationic liposomes**, and the role of p53 in the cellular response to DNA underlying the use of p53 gene therapy in combination with DNA-damaging agents (chemotherapeutics and **radiation**) are described.

REFERENCE COUNT: 139 THERE ARE 139 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 24 OF 26 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2002064455 EMBASE
TITLE: Effect of functional magnetic particles on radiofrequency capacitive heating: An in vivo study.
AUTHOR: Shinkai M.; Ueda K.; Ohtsu S.; Honda H.; Kohri K.; Kobayashi T.
CORPORATE SOURCE: T. Kobayashi, Department of Biotechnology, School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. takeshi@nubio.nagoya-u.ac.jp
SOURCE: Japanese Journal of Cancer Research, (2002) 93/1 (103-108). Refs: 16
ISSN: 0910-5050 CODEN: JJCREP
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 014 Radiology
016 Cancer
027 Biophysics, Bioengineering and Medical Instrumentation
033 Orthopedic Surgery
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Specific heating of magnetic particles in radiofrequency (RF) capacitive hyperthermia and its hyperthermic effect were investigated in an in vivo

study. Magnetite **cationic liposomes** (MCLs) were injected into a rat tumor on the femur and 8 MHz-RF capacitive heating was applied to the rat under 'mild heating' conditions. Although the input power of RF capacitive heating was low under the same power conditions, the MCLs-injected tumor was heated over 43.degree.C, whereas it was only heated to 41.degree.C in the case of the rats not injected with MCLs. A necrotic area in the tumor was observed in the heated rats. From the results of histological observation of the removed tissue, the necrotic area in the MCLs-injected tumor was wider than that in MCLs-free tumor. Complete tumor suppression was observed in 71% (5/7) of MCLs-injected rats, and the hyperthermic effect was greatly improved by the MCLs.

L8 ANSWER 25 OF 26 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998256605 EMBASE
TITLE: An inverted hexagonal phase of **cationic liposome**-DNA complexes related to DNA release and delivery.
AUTHOR: Koltover I.; Salditt T.; Radler J.O.; Safinya C.R.
CORPORATE SOURCE: C.R. Safinya, Materials Department, Physics Dept., Biochemistry/Molecular Biol. Program, University of California, Santa Barbara, CA 93106, United States
SOURCE: Science, (3 Jul 1998) 281/5373 (78-81).
Refs: 21
ISSN: 0036-8075 CODEN: SCIEAS
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A two-dimensional columnar phase in mixtures of DNA completed with **cationic liposomes** has been found in the lipid composition regime known to be significantly more efficient at transfecting mammalian cells in culture compared to the lamellar (L(.alpha.(C)) structure of **cationic liposome**-DNA complexes. The structure, derived from synchrotron x-ray diffraction, consists of DNA coated by cationic lipid minelayers and arranged on a two-dimensional hexagonal lattice (H(II)(C)). Two membrane-altering pathways induce the L(.alpha.)(C) .fwdarw. H((II)(C) transition: one where the spontaneous curvature of the lipid monolayer is driven negative, and another where the membrane binding rigidity is lowered with a new class of helper-lipids. Optical microscopy revealed that the L(.alpha.)(C) complexes bind stably to anionic vesicles (models of cellular membranes), whereas the more transfectant H(II)(C) complexes are unstable and rapidly fuse and release DNA upon adhering to anionic vesicles.

L8 ANSWER 26 OF 26 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97342674 EMBASE
DOCUMENT NUMBER: 1997342674
TITLE: Mechanism of adenovirus improvement of **cationic liposome**-mediated gene transfer.
AUTHOR: Meunier-Durmont C.; Picart R.; Ragot T.; Perricaudet M.; Hainque B.; Forest C.
CORPORATE SOURCE: C. Forest, Centre de Recherche, Endocrinol.Moleculaire/Developpement, CNRS UPR 9078, 9 rue Jules Hetzel, 92190 Meudon, France
SOURCE: Biochimica et Biophysica Acta - Biomembranes, (1997) 1330/1 (8-16).
Refs: 52
ISSN: 0005-2736 CODEN: BBBMBS
PUBLISHER IDENT.: S 0005-2736(97)00133-8
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics

029 Clinical Biochemistry
037 Drug Literature Index

LANGUAGE: English
SUMMARY LANGUAGE: English

AB Substantial effort has been focused on the development of highly efficient gene transfer strategies, Although viral and non-viral methods have been elaborated, mechanisms of gene delivery are still poorly understood. We exploited our recent observation that replication-deficient type 5 adenovirus dramatically enhances lipofectAMINE-mediated gene transfer (lipoadenofection) in differentiated cells to elucidate the mechanism of adenovirus action in this process. Heat-induced denaturation of viral capsid abolishes adenovirus action whereas inactivation of viral genome by short treatment with UV has no effect. Electron microscopic observations reveal the formation of a complex containing adenovirus and lipofectAMINE which probably carries DNA into cells via endocytosis. Anti-adenovirus antiserum or monoclonal anti-.alpha.(v).beta.3 integrin antibody inhibits lipoadenofection, at least partially. Neutralization of endosomal compartments with chloroquine, ammonium chloride or monensin does not prevent adenovirus improvement of gene transfer. Hence, adenovirus-lipofectAMINE-DNA complexes in which viral particles are each encompassed by three lipid layers, penetrate cells via an endocytic pathway involving probably the adenovirus receptor and .alpha.(v).beta.3 integrin. The resulting efficient transfer and expression of plasmid DNA proceeds from a mechanism in which adenoviral endosomolytic activity appears to be required while viral genome is not essential.

L9 ANSWER 1 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:367845 BIOSIS
DOCUMENT NUMBER: PREV200100367845
TITLE: Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm kinase.
AUTHOR(S): Macaulay, V. M. (1); Salisbury, A. J.; Bohula, E. A.; Playford, M. P.; Smorodinsky, N. I.; Shiloh, Y.
CORPORATE SOURCE: (1) IGF Group, Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, Oxford, OX3 9DS: macaulay@icrf.icnet.uk UK
SOURCE: Oncogene, (5 July, 2001) Vol. 20, No. 30, pp. 4029-4040. print.
ISSN: 0950-9232.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The type 1 insulin-like growth factor receptor (IGF1R) is required for growth, tumorigenicity and protection from apoptosis. IGF1R overexpression is associated with radioresistance in breast cancer. We used **antisense** (AS) RNA to downregulate IGF1R expression in mouse melanoma cells. Cells expressing AS-IGF1R transcripts were more **radiosensitive** in vitro and in vivo than controls. Also they showed reduced radiation-induced p53 accumulation and p53 serine 18 phosphorylation, and radioresistant DNA synthesis. These changes were reminiscent of the cellular phenotype of the human genetic disorder ataxia-telangiectasia (A-T), caused by mutations in the ATM gene. Cellular Atm protein levels were lower in AS-IGF1R-transfected cells than in control cells, although there was no difference in Atm expression at the transcriptional level. AS-IGF1R cells had detectable basal Atm kinase activity, but failed to induce kinase activity after irradiation. This suggests that IGF1R signalling can modulate the function of Atm, and supports the concept of targeted IGF1R downregulation as a potential treatment for malignant melanoma and other radioresistant tumours.

L9 ANSWER 2 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:322001 BIOSIS
DOCUMENT NUMBER: PREV200000322001
TITLE: Increased repair and cell survival in cells treated with DIR1 **antisense** oligonucleotides: Implications for induced radioresistance.
AUTHOR(S): Robson, T. (1); Price, M. E.; Moore, M. L.; Joiner, M. C.; McKelvey-Martin, V. J.; McKeown, S. R.; Hirst, D. G.
CORPORATE SOURCE: (1) Radiation Science Group, School of Biomedical Sciences, University of Ulster, Newtownabbey, Co. Antrim, BT37 OQB UK
SOURCE: International Journal of Radiation Biology, (May, 2000) Vol. 76, No. 5, pp. 617-623. print.
ISSN: 0955-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Purpose: To determine whether repression of a recently isolated, X-ray-responsive gene, DIR1, using **antisense** oligonucleotides could affect clonogenic cell survival and repair of DNA strand breaks and have a possible role in the mechanism underlying the phenomenon of 'induced radioresistance' (IRR). Materials and methods: Three cell lines, V79, RT112 and UM-UC-3, which are known to exhibit low-dose hypersensitivity (HRS) and induced radioresistance (IRR), and the **radiosensitive** cell line ATBIVA, were transfected with **antisense** oligonucleotides directed towards the DIR1 gene. Scrambled oligonucleotides were used as controls. DNA single-strand break (ssb) repair, using the alkaline comet assay, and cell survival using a standard clonogenic assay was measured after exposure to X-rays. Results:

Following treatment with 4 Gy X-rays, the V79, RT112 and UM-UC-3 cell lines all exhibited significantly increased rates of ssb repair after transfection with DIR1 **antisense** oligonucleotides compared with cells transfected with scrambled oligonucleotides. They also demonstrated significantly enhanced survival after exposure to 2 Gy X-rays; the **radiosensitive** ATBIVA cells did not show these effects. Conclusions: Repression of the DIR1 gene product leads to an increase in the rate of repair and cell survival in three radioresistant cells lines but not in the **radiosensitive** ATBIVA cell line. Because DIR1 is repressed by X-rays in the dose range where IRR is observed, it may represent a candidate gene involved in the IRR phenomenon.

L9 ANSWER 3 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:200834 BIOSIS
DOCUMENT NUMBER: PREV200000200834
TITLE: Transfer of Ku86 RNA **antisense** decreases the radioresistance of human fibroblasts.
AUTHOR(S): Marangoni, Elisabetta; Le Romancer, Muriel; Foray, Nicolas; Muller, Catherine; Douc-Rasy, Setha; Vaganay, Sabine; Abdulkarim, Bassam; Barrois, Michel; Calsou, Patrick; Bernier, Jacques; Salles, Bernard; Bourhis, Jean (1)
CORPORATE SOURCE: (1) Radiotherapie, Institut Gustave Roussy, 94805, Villejuif France
SOURCE: Cancer Gene Therapy, (Feb., 2000) Vol. 7, No. 2, pp. 339-346.
ISSN: 0929-1903.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Ku86 has been shown to be involved in DNA double-strand break (DSB) repair and radiosensitivity in rodents, but its role in human cells is still under investigation. The purpose of this study was to evaluate the radiosensitivity and DSB repair after transfection of a Ku86-**antisense** in a human fibroblast cell line. Simian virus 40-transformed MRC5V1 human fibroblasts were transfected with a vector (pcDNA3) containing a Ku86-**antisense** cDNA. The main endpoints were Ku86 protein level, Ku DNA end-binding and DNA protein kinase activity, clonogenic survival, and DSB repair kinetics. After transfection of the Ku86-**antisense**, decreased Ku86 protein expression, Ku DNA end-binding activity, and DNA protein kinase activity were observed in the uncloned cellular population. The fibroblasts transfected with the Ku86-**antisense** showed also a **radiosensitive** phenotype, with a surviving fraction at 2 Gy of 0.29 compared with 0.75 for the control and 20% of unrepaired DSB observed at 24 hours after irradiation compared with 0% for the control. Several clones were also isolated with a decreased level of Ku86 protein, a surviving fraction at 2 Gy between 0.05 and 0.40, and 10-20% of unrepaired DSB at 24 hours. This study is the first to show the implication of Ku86 in DSB repair and in the radiosensitivity of human cells. This investigation strongly suggests that Ku86 could constitute an appealing target for combining gene therapy and radiation therapy.

L9 ANSWER 4 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:433629 BIOSIS
DOCUMENT NUMBER: PREV199800433629
TITLE: An anti-sense construct of full-length ATM cDNA imposes a **radiosensitive** phenotype on normal cells.
AUTHOR(S): Zhang, Ning; Chen, Phil; Gatei, Magtouf; Scott, Shaun; Khanna, Kum Kum; Lavin, Martin F. (1)
CORPORATE SOURCE: (1) Queensland Cancer Fund Res. Lab., Queensland Inst. Med. Res., PO Royal Brisbane Hosp., Herston, Brisbane, QLD 4029 Australia
SOURCE: Oncogene, (Aug. 20, 1998) Vol. 17, No. 7, pp. 811-818.
ISSN: 0950-9232.
DOCUMENT TYPE: Article

LANGUAGE: English

AB The cloning of a full-length cDNA for the gene (ATM) mutated in the human genetic disorder ataxia-telangiectasia (A-T) has been described recently. This cDNA, as well as a fragment representing a functional region from ATM, are capable of rescuing various aspects of the **radiosensitive** phenotype in A-T cells. We have subcloned full-length ATM cDNA in the opposite orientation in an EBV-based vector under the control of an inducible promoter to determine whether this anti-sense construct might sensitize control lymphoblastoid cells to ionizing radiation. The effectiveness of expression of this construct in control cells was monitored by loss of ATM protein which was evident over a period 6-12 h after induction. Under these conditions radiosensitivity was enhanced approximately threefold in control cells, approaching the degree of radiosensitivity observed in A-T cells. Expression of the anti-sense construct also increased the number of radiation-induced chromosomal breaks and led to the appearance of radioresistant DNA synthesis in these cells. Abrogation of the G1/S checkpoint was evident from the loss of the p53 response and that of its downstream effector, p21/WAF1, post-irradiation. The extent of accumulation of transfected cells in G2/M phase at 24 h post-irradiation was similar to that observed in A-T cells and the induction of stress-activated protein kinase by ionizing radiation was prevented by **antisense** ATM cDNA expression. These data demonstrate that full-length ATM anti-sense cDNA, by reducing the amount of ATM protein, is effective in imposing a series of known defects characteristic of the A-T phenotype. This inducible system provides an experimental model to further investigate mechanisms underlying radiosensitivity and cell cycle control.

L9 ANSWER 5 OF 16 MEDLINE

ACCESSION NUMBER: 2001444708 MEDLINE

DOCUMENT NUMBER: 21385704 PubMed ID: 11494131

TITLE: Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm kinase.

AUTHOR: Macaulay V M; Salisbury A J; Bohula E A; Playford M P; Smorodinsky N I; Shiloh Y

CORPORATE SOURCE: IGF Group, Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, Oxford, OX3 9DS, UK.. macaulay@icrf.icnet.uk

CONTRACT NUMBER: R01 NS31763 (NINDS)

SOURCE: ONCOGENE, (2001 Jul 5) 20 (30) 4029-40. Journal code: 8711562. ISSN: 0950-9232.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200108

ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20010903
Entered Medline: 20010830

AB The type 1 insulin-like growth factor receptor (IGF1R) is required for growth, tumorigenicity and protection from apoptosis. IGF1R overexpression is associated with radioresistance in breast cancer. We used **antisense** (AS) RNA to downregulate IGF1R expression in mouse melanoma cells. Cells expressing AS-IGF1R transcripts were more **radiosensitive** in vitro and in vivo than controls. Also they showed reduced radiation-induced p53 accumulation and p53 serine 18 phosphorylation, and radioresistant DNA synthesis. These changes were reminiscent of the cellular phenotype of the human genetic disorder ataxia-telangiectasia (A-T), caused by mutations in the ATM gene. Cellular Atm protein levels were lower in AS-IGF1R-transfected cells than in control cells, although there was no difference in Atm expression at the transcriptional level. AS-IGF1R cells had detectable basal Atm kinase

activity, but failed to induce kinase activity after irradiation. This suggests that IGF1R signalling can modulate the function of Atm, and supports the concept of targeted IGF1R downregulation as a potential treatment for malignant melanoma and other radioresistant tumours.

L9 ANSWER 6 OF 16 MEDLINE
ACCESSION NUMBER: 2000385149 MEDLINE
DOCUMENT NUMBER: 20231414 PubMed ID: 10770645
TITLE: Transfer of Ku86 RNA **antisense** decreases the radioresistance of human fibroblasts.
AUTHOR: Marangoni E; Le Romancer M; Foray N; Muller C; Douc-Rasy S; Vaganay S; Abdulkarim B; Barrois M; Calsou P; Bernier J; Salles B; Bourhis J
CORPORATE SOURCE: Unite Propre de l'Enseignement Superieur Radiosensibilite-Radiocarcinogenese humaine, Institut Gustave Roussy, Villejuif, France.
SOURCE: CANCER GENE THERAPY, (2000 Feb) 7 (2) 339-46.
Journal code: 9432230. ISSN: 0929-1903.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20021001
Entered Medline: 20000810

AB Ku86 has been shown to be involved in DNA double-strand break (DSB) repair and radiosensitivity in rodents, but its role in human cells is still under investigation. The purpose of this study was to evaluate the radiosensitivity and DSB repair after transfection of a Ku86-**antisense** in a human fibroblast cell line. Simian virus 40-transformed MRC5V1 human fibroblasts were transfected with a vector (pcDNA3) containing a Ku86-**antisense** cDNA. The main endpoints were Ku86 protein level, Ku DNA end-binding and DNA protein kinase activity, clonogenic survival, and DSB repair kinetics. After transfection of the Ku86-**antisense**, decreased Ku86 protein expression, Ku DNA end-binding activity, and DNA protein kinase activity were observed in the uncloned cellular population. The fibroblasts transfected with the Ku86-**antisense** showed also a **radiosensitive** phenotype, with a surviving fraction at 2 Gy of 0.29 compared with 0.75 for the control and 20% of unrepaired DSB observed at 24 hours after irradiation compared with 0% for the control. Several clones were also isolated with a decreased level of Ku86 protein, a surviving fraction at 2 Gy between 0.05 and 0.40, and 10-20% of unrepaired DSB at 24 hours. This study is the first to show the implication of Ku86 in DSB repair and in the radiosensitivity of human cells. This investigation strongly suggests that Ku86 could constitute an appealing target for combining gene therapy and radiation therapy.

L9 ANSWER 7 OF 16 MEDLINE
ACCESSION NUMBER: 2000322652 MEDLINE
DOCUMENT NUMBER: 20322652 PubMed ID: 10866283
TITLE: Increased repair and cell survival in cells treated with DIR1 **antisense** oligonucleotides: implications for induced radioresistance.
AUTHOR: Robson T; Price M E; Moore M L; Joiner M C; McKelvey-Martin V J; McKeown S R; Hirst D G
CORPORATE SOURCE: Radiation Science Group, School of Biomedical Sciences, University of Ulster, Newtownabbey, N Ireland, UK.. T.Robson@Ulst.ac.uk
SOURCE: INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, (2000 May) 76 (5) 617-23.
Journal code: 8809243. ISSN: 0955-3002.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000713

AB PURPOSE: To determine whether repression of a recently isolated, X-ray-responsive gene, DIR1, using **antisense** oligonucleotides could affect clonogenic cell survival and repair of DNA strand breaks and have a possible role in the mechanism underlying the phenomenon of 'induced radioresistance' (IRR). MATERIALS AND METHODS: Three cell lines, V79, RT112 and UM-UC-3, which are known to exhibit low-dose hypersensitivity (HRS) and induced radioresistance (IRR), and the **radiosensitive** cell line ATBIVA, were transfected with **antisense** oligonucleotides directed towards the DIR1 gene. Scrambled oligonucleotides were used as controls. DNA single-strand break (ssb) repair, using the alkaline comet assay, and cell survival using a standard clonogenic assay was measured after exposure to X-rays. RESULTS: Following treatment with 4Gy X-rays, the V79, RT112 and UM-UC-3 cell lines all exhibited significantly increased rates of ssb repair after transfection with DIR1 **antisense** oligonucleotides compared with cells transfected with scrambled oligonucleotides. They also demonstrated significantly enhanced survival after exposure to 2 Gy X-rays; the **radiosensitive** ATBIVA cells did not show these effects. CONCLUSIONS: Repression of the DIR1 gene product leads to an increase in the rate of repair and cell survival in three radioresistant cells lines but not in the **radiosensitive** ATBIVA cell line. Because DIR1 is repressed by X-rays in the dose range where IRR is observed, it may represent a candidate gene involved in the IRR phenomenon.

L9 ANSWER 8 OF 16 MEDLINE
ACCESSION NUMBER: 1998451277 MEDLINE
DOCUMENT NUMBER: 98451277 PubMed ID: 9779997
TITLE: An anti-sense construct of full-length ATM cDNA imposes a **radiosensitive** phenotype on normal cells.
AUTHOR: Zhang N; Chen P; Gatei M; Scott S; Khanna K K; Lavin M F
CORPORATE SOURCE: Queensland Cancer Fund Research Laboratories, Brisbane, Australia.
SOURCE: ONCOGENE, (1998 Aug 20) 17 (7) 811-8.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123

AB The cloning of a full-length cDNA for the gene (ATM) mutated in the human genetic disorder ataxia-telangiectasia (A-T) has been described recently. This cDNA, as well as a fragment representing a functional region from ATM, are capable of rescuing various aspects of the **radiosensitive** phenotype in A-T cells. We have subcloned full-length ATM cDNA in the opposite orientation in an EBV-based vector under the control of an inducible promoter to determine whether this anti-sense construct might sensitize control lymphoblastoid cells to ionizing radiation. The effectiveness of expression of this construct in control cells was monitored by loss of ATM protein which was evident over a period 6-12 h after induction. Under these conditions radiosensitivity was enhanced approximately threefold in control cells, approaching the degree of radiosensitivity observed in A-T cells. Expression of the anti-sense construct also increased the number of radiation-induced chromosomal breaks and led to the appearance of radioresistant DNA synthesis in these cells. Abrogation of the G1/S checkpoint was evident from the loss of the

p53 response and that of its downstream effector, p21/WAF1, post-irradiation. The extent of accumulation of transfected cells in G2/M phase at 24 h post-irradiation was similar to that observed in A-T cells and the induction of stress-activated protein kinase by ionizing radiation was prevented by **antisense** ATM cDNA expression. These data demonstrate that full-length ATM anti-sense cDNA, by reducing the amount of ATM protein, is effective in imposing a series of known defects characteristic of the A-T phenotype. This inducible system provides an experimental model to further investigate mechanisms underlying radiosensitivity and cell cycle control.

L9 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:543451 CAPLUS
DOCUMENT NUMBER: 135:255307
TITLE: Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm kinase
AUTHOR(S): Macaulay, V. M.; Salisbury, A. J.; Bohula, E. A.; Playford, M. P.; Smorodinsky, N. I.; Shiloh, Y.
CORPORATE SOURCE: IGF Group, Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, Oxford, OX3 9DS, UK
SOURCE: Oncogene (2001), 20(30), 4029-4040
CODEN: ONCNES; ISSN: 0950-9232
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The type 1 insulin-like growth factor receptor (IGF1R) is required for growth, tumorigenicity and protection from apoptosis. IGF1R overexpression is assocd. with radioresistance in breast cancer. The authors used **antisense** (AS) RNA to downregulate IGF1R expression in mouse melanoma cells. Cells expressing AS-IGF1R transcripts were more **radiosensitive** in vitro and in vivo than controls. Also they showed reduced radiation-induced p53 accumulation and p53 serine 18 phosphorylation, and radioresistant DNA synthesis. These changes were reminiscent of the cellular phenotype of the human genetic disorder ataxia-telangiectasia (A-T), caused by mutations in the ATM gene. Cellular Atm protein levels were lower in AS-IGF1R-transfected cells than in control cells, although there was no difference in Atm expression at the transcriptional level. AS-IGF1R cells had detectable basal Atm kinase activity, but failed to induce kinase activity after irradn. This suggests that IGF1R signaling can modulate the function of Atm, and supports the concept of targeted IGF1R downregulation as a potential treatment for malignant melanoma and other radioresistant tumors.
REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:397861 CAPLUS
DOCUMENT NUMBER: 133:116762
TITLE: Increased repair and cell survival in cells treated with DIR1 **antisense** oligonucleotides: implications for induced radioresistance
AUTHOR(S): Robson, T.; Price, M. E.; Moore, M. L.; Joiner, M. C.; McKelvey-Martin, V. J.; McKeown, S. R.; Hirst, D. G.
CORPORATE SOURCE: Radiation Science Group, School of Biomedical Sciences, University of Ulster, Newtownabbey, Co. Antrim, BT37 OQB, UK
SOURCE: International Journal of Radiation Biology (2000), 76(5), 617-623
CODEN: IJRBE7; ISSN: 0955-3002
PUBLISHER: Taylor & Francis Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Purpose: To det. whether repression of a recently isolated, X-ray-responsive gene, DIR1, using **antisense** oligonucleotides could affect clonogenic cell survival and repair of DNA strand breaks and have a possible role in the mechanism underlying the phenomenon of "induced radioresistance" (IRR). Materials and methods: Three cell lines, V79, RT112 and UM-UC-3, which are known to exhibit low-dose hypersensitivity (HRS) and induced radioresistance (IRR), and the **radiosensitive** cell line ATBIVA, were transfected with **antisense** oligonucleotides directed towards the DIR1 gene. Scrambled oligonucleotides were used as controls. DNA single-strand break (ssb) repair, using the alk. comet assay, and cell survival using a std. clonogenic assay was measured after exposure to X-rays. Results: Following treatment with 4 Gy X-rays, the V79, RT112 and UM-UC-3 cell lines all exhibited significantly increased rates of ssb repair after transfection with DIR1 **antisense** oligonucleotides compared with cells transfected with scrambled oligonucleotides. They also demonstrated significantly enhanced survival after exposure to 2 Gy X-rays; the **radiosensitive** ATBIVA cells did not show these effects. Conclusions: Repression of the DIR1 gene product leads to an increase in the rate of repair and cell survival in three radioresistant cells lines but not in the **radiosensitive** ATBIVA cell line. Because DIR1 is repressed by X-rays in the dose range where IRR is obsd., it may represent a candidate gene involved in the IRR phenomenon.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:228338 CAPLUS
DOCUMENT NUMBER: 132:331430
TITLE: Transfer of Ku86 RNA **antisense** decreases the radioresistance of human fibroblasts
AUTHOR(S): Marangoni, Elisabetta; Le Romancer, Muriel; Foray, Nicolas; Muller, Catherine; Douc-Rasy, Setha; Vaganay, Sabine; Abdulkarim, Bassam; Barrois, Michel; Calsou, Patrick; Bernier, Jacques; Salles, Bernard; Bourhis, Jean
CORPORATE SOURCE: Unite Propre de l'Enseignement Superieur "Radiosensibilite-Radiocarcinogenese humaine", Institut Gustave Roussy, Villejuif, 94805, Fr.
SOURCE: Cancer Gene Therapy (2000), 7(2), 339-346
CODEN: CGTHEG; ISSN: 0929-1903
PUBLISHER: Nature America, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ku86 has been shown to be involved in DNA double-strand break (DSB) repair and radiosensitivity in rodents, but its role in human cells is still under investigation. The purpose of this study was to evaluate the radiosensitivity and DSB repair after transfection of a Ku86-**antisense** in a human fibroblast cell line. Simian virus 40-transformed MRC5V1 human fibroblasts were transfected with a vector (pcDNA3) contg. a Ku86-**antisense** cDNA. The main endpoints were Ku86 protein level, Ku DNA end-binding and DNA protein kinase activity, clonogenic survival, and DSB repair kinetics. After transfection of the Ku86-**antisense**, decreased Ku86 protein expression, Ku DNA end-binding activity, and DNA protein kinase activity were obsd. in the uncloned cellular population. The fibroblasts transfected with the Ku86-**antisense** showed also a **radiosensitive** phenotype, with a surviving fraction at 2 Gy of 0.29 compared with 0.75 for the control and 20% of unrepaired DSB obsd. at 24 h after irradn. compared with 0% for the control. Several clones were also isolated with a decreased level of Ku86 protein, a surviving fraction at 2 Gy between 0.05 and 0.40, and 10-20% of unrepaired DSB at 24 h. This study is the first to show the implication of Ku86 in DSB repair and in the radiosensitivity of human cells. This investigation strongly suggests that Ku86 could constitute an appealing

target for combining gene therapy and radiation therapy.
REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:575325 CAPLUS
DOCUMENT NUMBER: 129:272359
TITLE: An anti-sense construct of full-length ATM cDNA imposes a **radiosensitive** phenotype on normal cells
AUTHOR(S): Zhang, Ning; Chen, Phil; Gatei, Magtouf; Scott, Shaun; Khanna, Kum Kum; Lavin, Martin F.
CORPORATE SOURCE: Queensland Cancer Fund Research Laboratories, Brisbane, 4029, Australia
SOURCE: Oncogene (1998), 17(7), 811-818
CODEN: ONCNES; ISSN: 0950-9232
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cloning of a full-length cDNA for the gene (ATM) mutated in the human genetic disorder ataxia-telangiectasia (A-T) has been described recently. This cDNA, as well as a fragment representing a functional region from ATM, are capable of rescuing various aspects of the **radiosensitive** phenotype in A-T cells. We have subcloned full-length ATM cDNA in the opposite orientation in an EBV-based vector under the control of an inducible promoter to det. whether this anti-sense construct might sensitize control lymphoblastoid cells to ionizing radiation. The effectiveness of expression of this construct in control cells was monitored by loss of ATM protein which was evident over a period 6-12 h after induction. Under these conditions radiosensitivity was enhanced approx. threefold in control cells, approaching the degree of radiosensitivity obsd. in A-T cells. Expression of the anti-sense construct also increased the no. of radiation-induced chromosomal breaks and led to the appearance of radioresistant DNA synthesis in these cells. Abrogation of the G1/S checkpoint was evident from the loss of the p53 response and that of its downstream effector, p21/WAF1, post-irradn. The extent of accumulation of transfected cells in G2/M phase at 24 h post-irradn. was similar to that obsd. in A-T cells and the induction of stress-activated protein kinase by ionizing radiation was prevented by **antisense** ATM cDNA expression. These data demonstrate that full-length ATM anti-sense cDNA, by reducing the amt. of ATM protein, is effective in imposing a series of known defects characteristic of the A-T phenotype. This inducible system provides an exptl. model to further investigate mechanisms underlying radiosensitivity and cell cycle control.

L9 ANSWER 13 OF 16 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001262831 EMBASE
TITLE: Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells is associated with enhanced radiosensitivity and impaired activation of Atm kinase.
AUTHOR: Macaulay V.M.; Salisbury A.J.; Bohula E.A.; Playford M.P.; Smorodinsky N.I.; Shiloh Y.
CORPORATE SOURCE: V.M. Macaulay, IGF Group, Molecular Oncology Laboratories, Weatherall Inst. of Molec. Medicine, Oxford OX3 9DS, United Kingdom. macaulay@icrf.icnet.uk
SOURCE: Oncogene, (5 Jul 2001) 20/30 (4029-4040).
Refs: 76
ISSN: 0950-9232 CODEN: ONCNES
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
029 Clinical Biochemistry

LANGUAGE: English
SUMMARY LANGUAGE: English
AB The type 1 insulin-like growth factor receptor (IGF1R) is required for growth, tumorigenicity and protection from apoptosis. IGF1R overexpression is associated with radioresistance in breast cancer. We used **antisense** (AS) RNA to downregulate IGF1R expression in mouse melanoma cells. Cells expressing AS-IGF1R transcripts were more **radiosensitive** in vitro and in vivo than controls. Also they showed reduced radiation-induced p53 accumulation and p53 serine 18 phosphorylation, and radioresistant DNA synthesis. These changes were reminiscent of the cellular phenotype of the human genetic disorder ataxia-telangiectasia (A-T), caused by mutations in the ATM gene. Cellular Atm protein levels were lower in AS-IGF1R-transfected cells than in control cells, although there was no difference in Atm expression at the transcriptional level. AS-IGF1R cells had detectable basal Atm kinase activity, but failed to induce kinase activity after irradiation. This suggests that IGF1R signalling can modulate the function of Atm, and supports the concept of targeted IGF1R downregulation as a potential treatment for malignant melanoma and other radioresistant tumours.

L9 ANSWER 14 OF 16 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000198173 EMBASE
TITLE: Increased repair and cell survival in cells treated with DIR1 **antisense** oligonucleotides: Implications for induced radioresistance.
AUTHOR: Robson T.; Price M.E.; Moore M.L.; Joiner M.C.; McKelvey-Martin V.J.; McKeown S.R.; Hirst D.G.
CORPORATE SOURCE: T. Robson, Radiation Science Group, School of Biomedical Sciences, University of Ulster, Newtownabbey, Co Antrim BT37 0QB, United States. T.Robson@Ulst.ac.uk
SOURCE: International Journal of Radiation Biology, (2000) 76/5 (617-623).
Refs: 24
ISSN: 0955-3002 CODEN: IJRBA3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 014 Radiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Purpose: To determine whether repression of a recently isolated, X-ray-responsive gene, DIR1, using **antisense** oligonucleotides could affect clonogenic cell survival and repair of DNA strand breaks and have a possible role in the mechanism underlying the phenomenon of induced radioresistance' (IRR). Materials and methods: Three cell lines, V79, RT112 and UM-UC-3, which are known to exhibit low-dose hypersensitivity (HRS) and induced radioresistance (IRR), and the **radiosensitive** cell line ATBIVA, were transfected with **antisense** oligonucleotides directed towards the DIR1 gene. Scrambled oligonucleotides were used as controls, DNA single-strand break (ssb) repair, using the alkaline comet assay, and cell survival using a standard clonogenic assay was measured after exposure to X-rays. Results: Following treatment with 4 Gy X-rays, the V79, RT112 and UM-UC-3 cell lines all exhibited significantly increased rates of ssb repair after transfection with DIR1 **antisense** oligonucleotides compared with cells transfected with scrambled oligonucleotides. They also demonstrated significantly enhanced survival after exposure to 2 Gy X-rays; the **radiosensitive** ATBIVA cells did not show these effects. Conclusions: Repression of the DIR1 gene product leads to an increase in the rate or repair and cell survival in three radioresistant cells lines but not in the **radiosensitive** ATBIVA cell line. Because DIR1 is repressed by X-rays in the dose range where IRR is observed, it may represent a candidate gene involved in the IRR phenomenon.

L9 ANSWER 15 OF 16 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000130276 EMBASE
TITLE: Transfer of Ku86 RNA **antisense** decreases the radioresistance of human fibroblasts.
AUTHOR: Marangoni E.; Le Romancer M.; Foray N.; Muller C.; Douc-Rasy S.; Vaganay S.; Abdulkarim B.; Barrois M.; Calsou P.; Bernier J.; Salles B.; Bourhis J.
CORPORATE SOURCE: Dr. J. Bourhis, Radiotherapie, Institut Gustave Roussy, 94805 Villejuif, France. bourhis@igr.fr
SOURCE: Cancer Gene Therapy, (2000) 7/2 (339-346).
Refs: 58
ISSN: 0929-1903 CODEN: CGTHEG
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 014 Radiology
016 Cancer
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Ku86 has been shown to be involved in DNA double-strand break (DSB) repair and radiosensitivity in rodents, but its role in human cells is still under investigation. The purpose of this study was to evaluate the radiosensitivity and DSB repair after transfection of a Ku86-**antisense** in a human fibroblast cell line. Simian virus 40-transformed MRC5V1 human fibroblasts were transfected with a vector (pcDNA3) containing a Ku86- **antisense** cDNA. The main endpoints were Ku86 protein level, Ku DNA end- binding and DNA protein kinase activity, clonogenic survival, and DSB repair kinetics. After transfection of the Ku86-**antisense**, decreased Ku86 protein expression, Ku DNA end-binding activity, and DNA protein kinase activity were observed in the uncloned cellular population. The fibroblasts transfected with the Ku86-**antisense** showed also a **radiosensitive** phenotype, with a surviving fraction at 2 Gy of 0.29 compared with 0.75 for the control and 20% of unrepaired DSB observed at 24 hours after irradiation compared with 0% for the control. Several clones were also isolated with a decreased level of Ku86 protein, a surviving fraction at 2 Gy between 0.05 and 0.40, and 10-20% of unrepaired DSB at 24 hours. This study is the first to show the implication of Ku86 in DSB repair and in the radiosensitivity of human cells. This investigation strongly suggests that Ku86 could constitute an appealing target for combining gene therapy and radiation therapy.

L9 ANSWER 16 OF 16 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998315903 EMBASE
TITLE: An anti-sense construct of full-length ATM cDNA imposes a **radiosensitive** phenotype on normal cells.
AUTHOR: Zhang N.; Chen P.; Gatei M.; Scott S.; Khanna K.K.; Lavin M.F.
CORPORATE SOURCE: M.F. Lavin, Queensland Cancer Fund Research Lab., PO Royal Brisbane Hospital, Herston, Brisbane, QLD 4029, Australia
SOURCE: Oncogene, (20 Aug 1998) 17/7 (811-818).
Refs: 66
ISSN: 0950-9232 CODEN: ONCNES
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The cloning of a full-length cDNA for the gene (ATM) mutated in the human genetic disorder ataxia-telangiectasia (A-T) has been described recently. This cDNA, as well as a fragment representing a functional region from ATM, are capable of rescuing various aspects of the **radiosensitive** phenotype in A-T cells. We have subcloned full-length ATM cDNA in the

opposite orientation in an EBV-based vector under the control of an inducible promoter to determine whether this anti-sense construct might sensitize control lymphoblastoid cells to ionizing radiation. The effectiveness of expression of this construct in control cells was monitored by loss of ATM protein which was evident over a period 6-12 h after induction. Under these conditions radiosensitivity was enhanced approximately threefold in control cells, approaching the degree of radiosensitivity observed in A-T cells. Expression of the anti-sense construct also increased the number of radiation-induced chromosomal breaks and led to the appearance of radioresistant DNA synthesis in these cells. Abrogation of the G1/S checkpoint was evident from the loss of the p53 response and that of its downstream effector, p21/WAF1, post-irradiation. The extent of accumulation of transfected cells in G2/M phase at 24 h post-irradiation was similar to that observed in A-T cells and the induction of stress-activated protein kinase by ionizing radiation was prevented by **antisense** ATM cDNA expression. These data demonstrate that full-length ATM anti-sense cDNA, by reducing the amount of ATM protein, is effective in imposing a series of known defects characteristic of the A-T phenotype. This inducible system provides an experimental model to further investigate mechanisms underlying radiosensitivity and cell cycle control.